Alimentary canal of fifth instar larvae of *Lymantria dispar* (Lepidoptera: Erebidae, Lymatriinae)

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Here, the alimentary canal of fifth instar larvae of the gypsy moth (*Lymantria dispar*) was re-investigated with light microscopy (LM), and for the first time with laser scanning confocal microscopy (LSCM) and scanning electron microscopy (SEM). We describe morphological characteristics and provide morphometric data. Well-developed dilator muscles and Malpighian bladders suggest a high capacity for food propulsion, excretion, absorption, and detoxification. The epithelium of the alimentary canal of *L. dispar* is histologically simple and that of the midgut is composed of columnar, goblet, and regenerative cells. Particular arrangement of the intima of crop with a central ventral region recalling a ladder and numerous folds was detected, indicating the capability for a large increase in volume to store food. Numerous spinules were observed on the cuticular intima of pylorus and spinules of a different type were observed on the pyloric valve, and these might assist in propelling the feces.

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1. Introduction

The gypsy moth, *Lymantria dispar* (Linnaeus, 1758), is considered one of the globally most devastating Lepidopteran defoliator pests (Vanhanen *et al.* 2007). The outbreaks of *L. dispar* pose considerable threats to ecosystems and cause substantial economic losses (Mayo *et al.* 2003). In particular, the species can have a strong impact on forest stand dynamics (Rafes 1970, Kozlowski *et al.* 1991) through severe damage to tree growth, frequently resulting in high mortality (Mark *et al.* 2007). As the larvae feed on the foliage of hundreds of plant species (Liebhold *et al.* 1992, Rich-

ards & Davies 1994), outbreaks of *L. dispar* can lead to the loss of valuable tree species, aesthetic damage, loss of wildlife habitat, and have a negative impact on the quality of surface water in affected areas (Eshleman *et al.* 2000, Potter *et al.* 2000). Great efforts are made to control *L. dispar*, and an effective program for the biological control of *L. dispar* uses the nucleopolyhedrovirus to enter the insect through the alimentary canal (Xu 2005).

In addition to numerous studies on the ecology and management of the species, some research attention has been paid to the morphology of *L. dispar* larvae, including the alimentary canal. Helm (1876) described the morphology and histology of the silk glands of *L. dispar* larvae. Wistinghausen (1890) described and illustrated the tracheal terminations on their silk glands. Kirkland (1896) gave a brief, illustrated description of the alimentary canal, silk glands, malpighian tubules, heart, central nervous system, tracheal system, fat body, and developing genitalia of *L. dispar* larvae. Klatt (1909) described the morphology and histology of the Verson's glands (including those which are modified into funnel warts) of gypsy moth larvae. Moreover, in order to explain the mechanism of protrusion of the funnel warts, he also described and illustrated the musculature of the dorsal body wall of their 5th and 6th abdominal segments. The funnel warts of *L. dispar* larvae were further treated in numerous other papers, including recent ones (e.g. Deml & Dettner 2001). Pospielow (1911, cited by Hufnagel 1918) described the oenocytes in moulting larvae of *L. dispar*. Ishimori (1924) described and illustrated the morphology of the cryptonephridial part of the Malpighian tubules of *L. dispar* larvae, whereas Dauberschmidt (1934) illustrated their basal portion. Lee (1948) described the morphology and innervation of the endocrine prothoracic glands of *L. dispar* larvae. Traxler (1977) described and illustrated the morphology of gypsy moth larvae, including a few internal structures, like the inner aspect of the cuticle of the head capsule and the gross morphology of the silk glands. Davis *et al.* (1989) illustrated the central nervous system and retrocerebral complex (corpus cardiacum, corpus allatum, and their nerves) of *L. dispar* larvae. Miller (1991) and Speidel *et al.* (1996) reported the absence of a ventral cervical gland (= adenosma) in larvae of the gypsy moth. Klein *et al.* (1999) described the morphology, histology, and ultrastructure of the epitracheal glands of *L. dispar* larvae. However, a detailed study of the alimentary canal is still needed to have comprehensive picture of its structure.

The morphology, histology, and physiology of the alimentary canal of arthropods are related to the digestion and absorption of their specific diet. Larvae of *L. dispar* are polyphagous on leaves of gymnosperms and angiosperms (Fernald 1896, Robinson *et al.* 2010), and the midgut, which occupies the largest part of the alimentary canal, plays a major role in the absorption of nutrients, but also of chemical and biological insecticides (Anderson *et al.* 1966, Keating *et al.* 1990, Pinheiro *et al.* 2003, Levy *et al.* 2004, 2008).

Despite a vast amount of literature on this insect species, internal ultramorphology of *L. dispar* needs further study. Our study provides the first detailed morphological description of the alimentary canal of *L. dispar* larvae and of the histology of the Malpighian tubules.

2. Materials and methods

Larvae of *L. dispar* were reared on an artificial diet in the laboratory and maintained under controlled temperature 25 °C, photoperiod (8h light/16h dark), and 40–50% relative humidity. For morphological analysis, over 20 larvae of the 5th instar (about 30 days of development) were killed by placing them in 100% ethanol. Using two fine forceps, the larvae were dissected in Petri dishes containing phosphate buffer solution (0.1 M, pH 7.4) under an Olympus SZX16 stereoscopic microscope, with the alimentary canal stained with methylrosanilinium chloride (Wang *et al.* 2009) (a clinical solution, comprising 1% methyl violet, 99% ethanol and purified water). A Canon 500D digital camera and a stereoscopic microscope were used to take a series of photographs with varying focus, and Helicon Focus for Windows (Helicon Soft Ltd, Kharkov, Ukraine) was used to compose those images with greater depth of field. In addition, Adobe Photoshop was applied with proportionate scale bars to get the measurements of morphological structures.

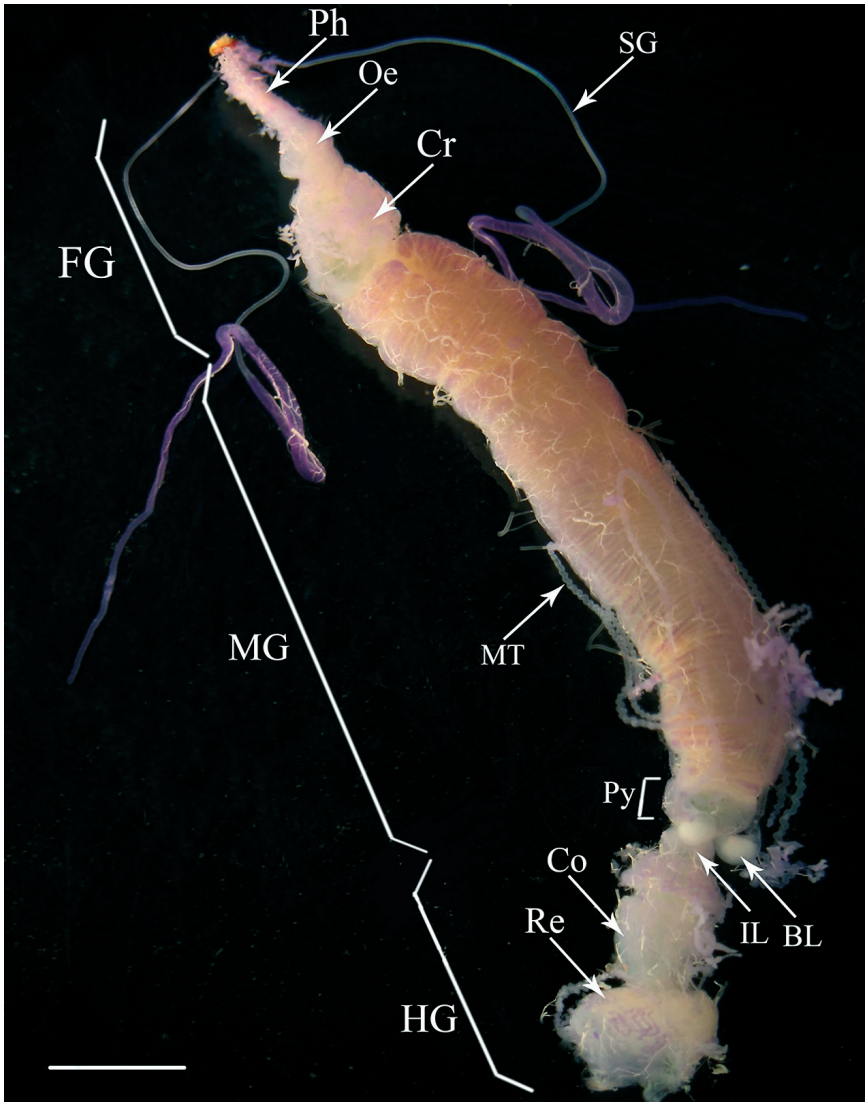

Fig. 1. Gross morphology of alimentary canal of fifth instar larvae of *Lymantria dispar*. Abbreviations: BL, bladder; Co, colon; Cr, crop; FG, foregut; HG, hindgut; IL, ileum; MG, midgut; MT, Malpighian tubule; Oe, esophagus; Ph, pharynx; Py, pylorus; Re, rectum; SG, silk gland. Scale bar: 5 mm.

For observations of general histology of the epithelial cells, the alimentary canal was isolated and fixed in 2.5% glutaraldehyde in phosphate buffer solution (pH 7.4, at 4 °C for 24 h). After dehydration in ethanol series (30, 50, 70, 80, 90, 95, and 100%, in each case for 1 h), the materials were embedded in paraffin wax using xylene as intermediate.

Continuous series of longitudinal sections were mounted on slides coated with poly-L-lysine, and stained with hematoxylin-eosin. Then, paraffin sections were observed under a LEICA DM I4000B (Leica Microsystems GmbH, Wetzlar, Germany) light microscope (LM). Subsequently, a LEICA TCS SP5 (Leica Microsystems GmbH, Wetzlar, Germany) laser

Table 1. Means ± SD of lengths and widths (mm) of different parts of alimentary canal of *Lymantria dispar* (*n* = 5).

Part	Length	Width
Entire gut	39.8 ± 4.5	—
Foregut	8.8 ± 1.5	—
Midgut	22.6 ± 2.4	4.3 ± 0.7
Hindgut	9.1 ± 1.5	—
Silk gland (Left)	42.3 ± 0.3	—

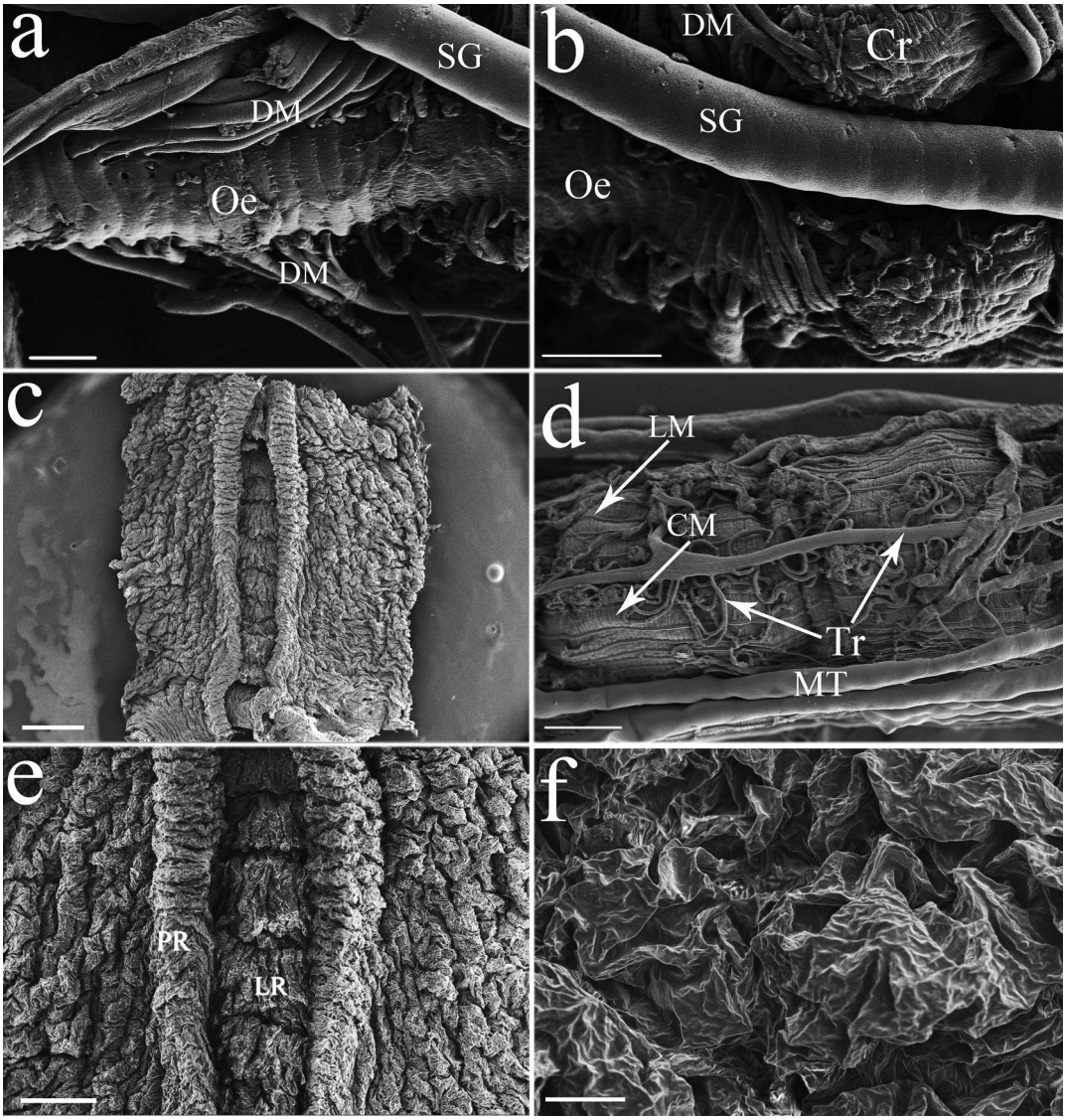

Fig. 2. SEM micrographs of foregut and midgut of fifth instar larvae of *Lymantria dispar*. – a. Well-developed dilator muscles attached to esophagus walls. – b. Ventral view of crop-midgut, showing sac-shaped crop. – c. Ventral view of cuticular intima of "unrolled" crop, showing central ventral regularly arranged region similar in form to a "ladder" and the surrounding folded region. – d. Ventral view of midgut, showing arrangement of outer longitudinal and inner circular muscles, and abundant tracheae of different sizes embedded in midgut. – e. Magnification of the "ladder" in (c), showing bilateral prominent parallel ridges and middle sectionalized longitudinal region. – f. Higher magnification of the folded intima of the crop, showing the rough surface. Abbreviations: CM, circular muscular layer; Cr, crop; DM, dilator muscles; LR, longitudinal region; LM, longitudinal muscular layer; MT, Malpighian tubule; Oe, esophagus; PR, parallel ridge; SG, silk gland; Tr, tracheae. Scale bars: 80 μm in a, 120 μm in b, 400 μm in c, 200 um in d and e, 20 μm in f.

scanning confocal microscope (LSCM) was applied to investigate the histological sections stained with hematoxylin-eosin in order to obtain higher resolution of epithelial cells under laser wave of 488 nm. The autofluorescence signal was collected in PMT channel with the collection windows of 510–535 nm (green).

For scanning electron microscope (SEM)

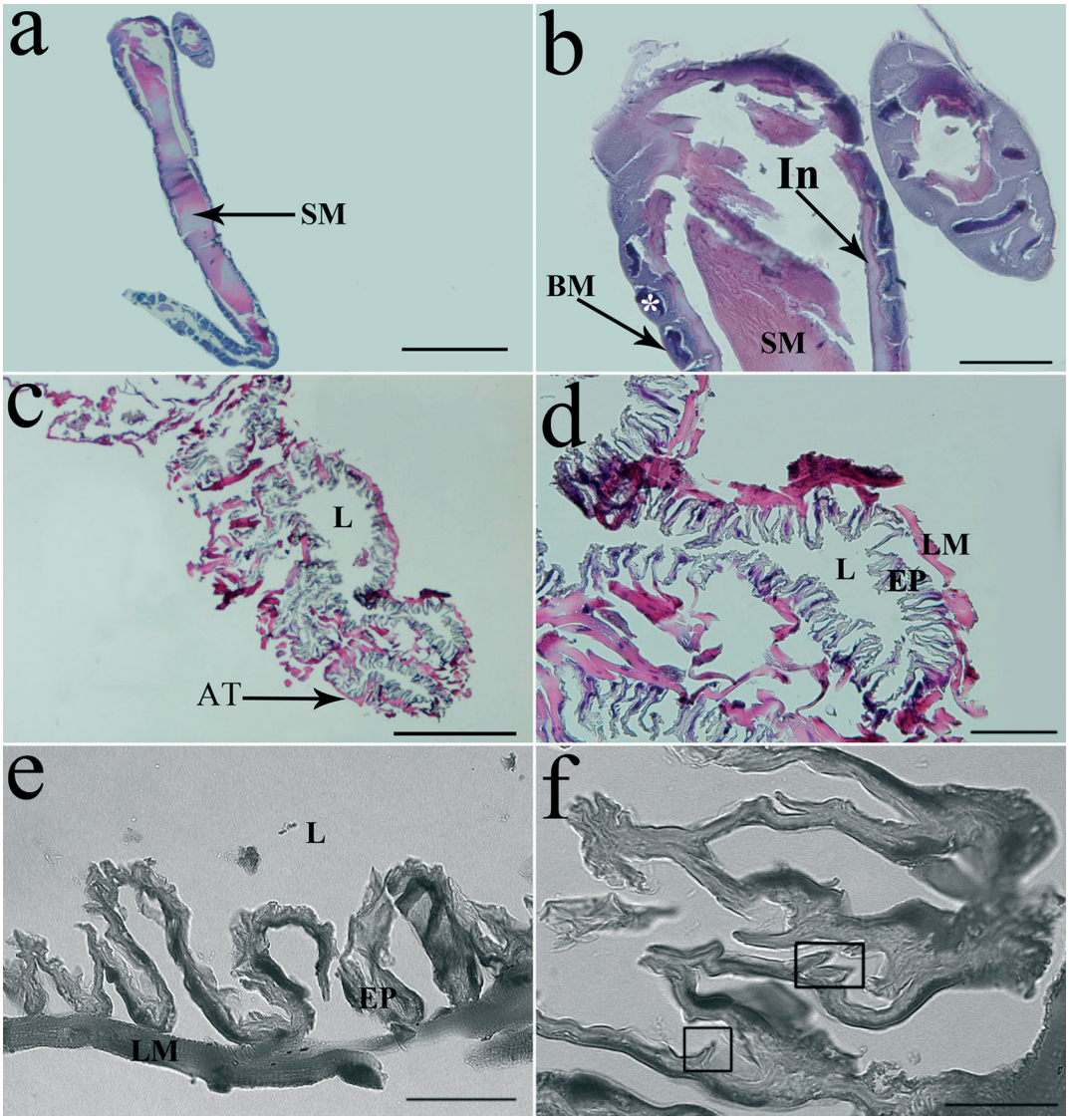

Fig. 3. Silk glands and crop of fifth instar larvae of *Lymantria dispar*. – a. Gross view of silk gland. – b. Magnification of silk gland showing intima and basement membrane and cells with prominent nuclei (white *). – c. Longitudinal section of crop. – d. Magnification of anterior region of crop, showing deeply folded epithelium. – e. Confocal micrograph of epithelium, showing flattened epithelial cells. – f. Magnification of epithelium, showing intima with spinous protuberances (in boxed region). Abbreviations: AT, anterior region; BM, basement membrane; EP, epithelium; In, intima; L, lumen; LM, longitudinal muscular layer; SM, silk mass. Scale bars: 500 µm in a and c, 100 µm in b and d, 50 µm in e, 25 µm in f.

studies, the alimentary canal was dehydrated in a graded ethanol series (30, 50, 70, 80, 90% and absolute ethanol for 1 h in each concentration). After having been dried by critical point method, the materials were coated with gold, analyzed and photographed by a HITACHI S3400 SEM (Hitachi Corp., Tokyo, Japan) at the Microscopy Core Facility, Biological Technology Center, Beijing Forestry University (Beijing, China). In order to view the inner side of the gut with the SEM, before dehydration the eye scissors were used carefully to dissect the gut, and then the gut contents were washed away by phosphate buffer solution (0.1 M, pH 7.4) using a dropper.

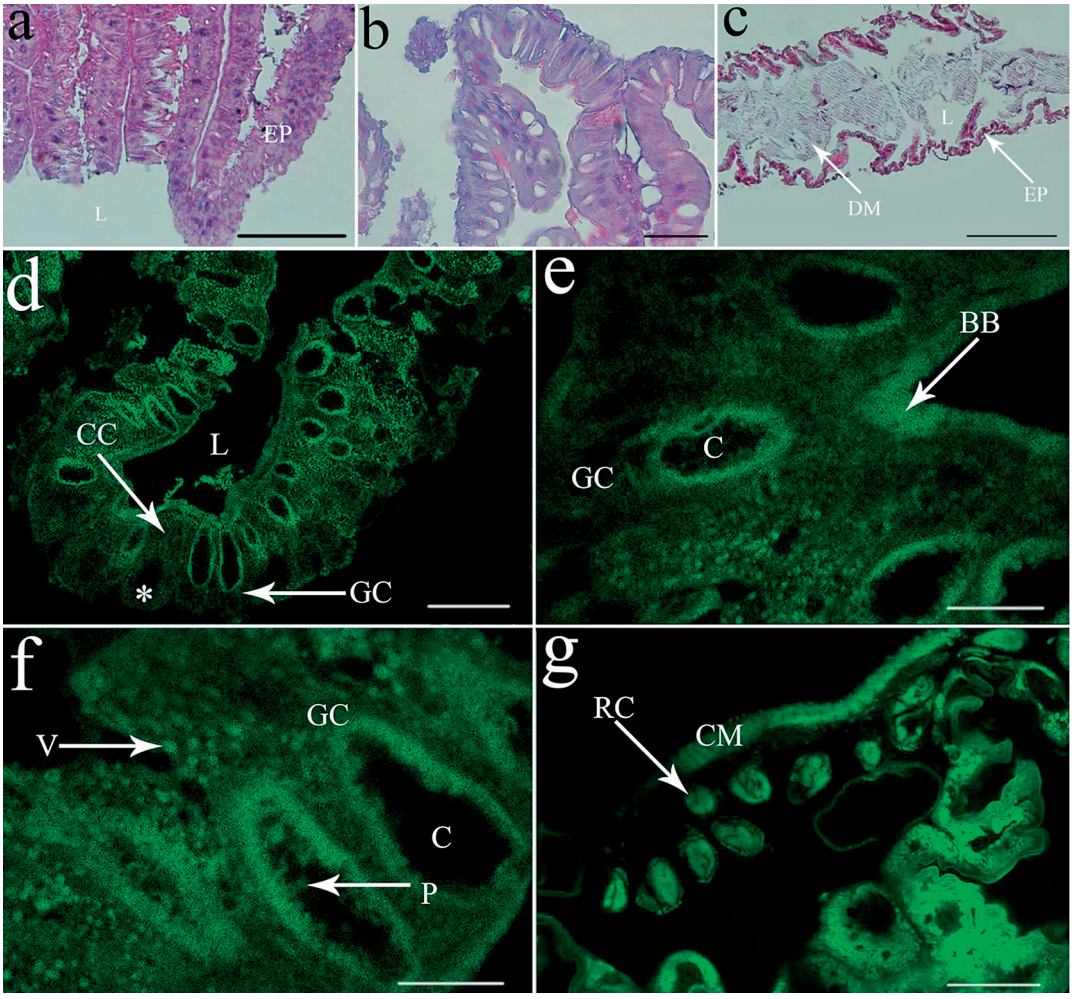

Fig. 4. Anterior midgut of fifth instar larvae of *Lymantria dispar*. – a. Deeply folded epithelium. – b. Light micrograph of epithelium showing goblet cells and column cells. – c. General view of anterior midgut, showing digested material and epithelium. – d. Confocal micrograph of epithelium showing goblet cells and columnar cells with oval nuclei (Asterisk). – e. Magnification of epithelium showing brush-shaped border and goblet cells with goblet-shaped cavity. – f. Magnification of epithelium showing goblet cells with numerous vacuoles at bottom and cytoplasmic projections in goblet-shaped cavity. – g. Higher magnification of regenerative cells with oval nuclei. Abbreviations: BB, brush border; C, globlet chamber; CC, columnar cell; CM, circular muscular layer; DM, digested material; EP, epithelium; GC, goblet cell; L, lumen; P, plasmatic membrane projections; RC, regenerative cell. Scale bars: 100 µm in a, 50 µm in b, 200 µm in c, 25 µm in d, 10 µm in e, 7.5 µm in f, 15 µm in g.

3. Anatomy of alimentary canal of fifth instar larvae of *L. dispar*

The current study combined the use of light microscopy, confocal microscopy, and SEM to provide a more detailed examination of the anatomy of the alimentary canal of the fifth instar larvae of *L. dispar*. The alimentary canal consists of the foregut, midgut, hindgut, and accessory organs, such as silk glands and Malpighian tubules, the latter projecting from the main digestive tube. The foregut and midgut are delimited by the cardiac valve, and the Malpighian tubules open in the alimentary canal shortly posterior to the border-line between midgut and hindgut. The foregut and hindgut are relatively short, while the midgut occupies almost the entire alimentary canal (Fig. 1). Measurements of the regions of the

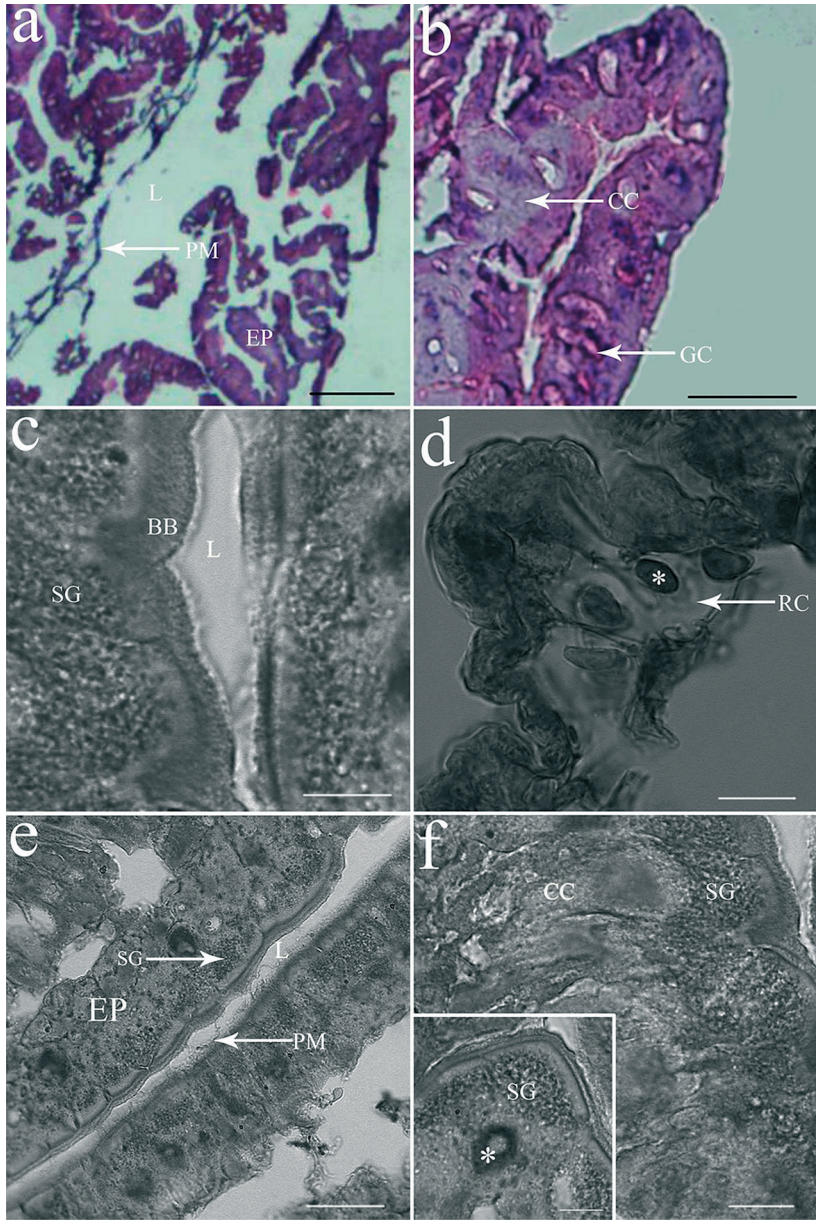

Fig. 5. Median and posterior midgut of fifth instar larvae of *Lymantria dispar*. – a. General view of epithelium, showing peritrophic membrane in midgut lumen. – b. Magnification of epithelium. – c. Confocal micrograph of epithelium showing numerous secretory granules in columnar cells with brush border in apical portion. – d. Regenerative cells with oval nuclei (white *) in cluster. – e. General view of epithelium under confocal laser scanning microscope. – f. Magnification of columnar cells showing numerous secretory granules and oval nuclei (white *). Abbreviations: BB, brush border; CC, columnar cell; EP, epithelium; GC, goblet cell; L, lumen; PM, peritrophic membrane; RC, regenerative cell; SG, secretory granules. Scale bars: 125 µm in a, 50 µm in b, 7.5 µm in c, 10 µm in d, 25 µm in e, 10 µm in f (7.5 µm in box).

alimentary canal and of the silk glands are shown in Table 1.

3.1. Foregut

The foregut consists sequentially of cibarium, pharynx, esophagus, crop, and the anterior portion of the cardiac valve (Fig. 1). The musculature of esophagus is composed of an external circular layer and lateral dilator muscles (Fig. 2a, b). Similar features have been described for other Lepidoptera (Drecktrah *et al.* 1966, Eaton 1988, Levy *et al.* 2008), Coleoptera (Areekul 1957, Vasques 1988) and Diptera (Patil & Govindan 1984). The dilator muscles of the fifth instar larvae of *L. dispar* are well-developed (Fig. 2a, b). The anatomical structure of the foregut of insects can be linked to their feeding habits: if they feed on solid material, as is the case for lepidopteran larvae, Blatta-

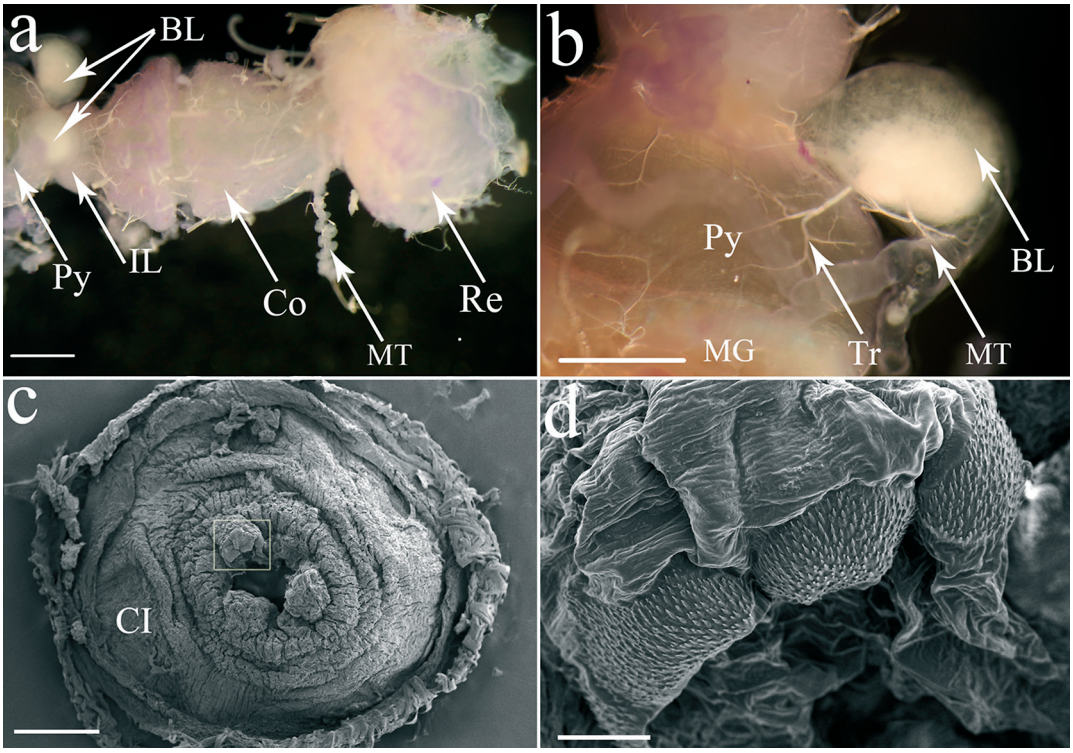

Fig. 6. Hindgut and Malpighian tubules of fifth instar larvae of *Lymantria dispar*. – a. Ventral view showing complex components of hindgut. – b. Magnification of midgut and pylorus, showing large bladder and tubules arising from it. – c. SEM micrograph of cross section of pylorus. – d. Magnification of boxed region in (c) showing armature of spinules on pyloric valve. Abbreviations: BL, bladder; CI, cuticular intima; Co, colon; IL, ileum; MG, midgut; MT, Malpighian tubule; Py, pylorus; Re, rectum; Tr, tracheae. Scale bars: 1 mm in a, 500 µm in b, 400 µm in c, 40 µm in d.

ria and Orthoptera, variations in the foregut diameter due to the circular and dilator muscles would be responsible for the food propulsion. In sucking insects these muscles are more developed and specialized to allow this region to act as a sucking pump for the food (Gillott 1995). Thus, a strong ability of *L. dispar* for food propulsion can be hypothesized.

3.2. Crop

The pear-shaped crop is a simple dilation of the posterior region of the esophagus, occupying the widest region of the foregut and being surrounded by muscular layers (Figs. 1, 2b, 3d, e). The functions of the crop are the storage and flow of ingested food (Wigglesworth 1984, Brusca & Brusca 1990, Chapman 1998, Gullan & Cranston 2009). The cuticular intima of "unrolled" crop consists of the surrounding deeply folded region and a central ventral regularly arranged region recalling a "ladder" (Fig. 2c), which is formed by bilateral prominent parallel ridges enclosing a middle sectionalized longitudinal region (Fig. 2e).

The surface of the folded intima of the crop is relatively rough (Fig. 2f), and the epithelium is deeply folded (Fig. 3c, d), permitting a large increase in volume to store food. The epithelial wall of the crop is formed of irregularly shaped flattened epithelial cells with indistinct margins (Fig. 3e), and the intima of epithelium forms into some spinous protuberances (Fig. 3f). The circular muscular fibers cannot be readily observed, differing from the condition in larvae of Anticarsia gemmatalis Hübner, for which clearly visible circular muscular fibers have been reported (Levy *et al.* 2008). This particular formation of the muscular layers differentiates the crop region from the

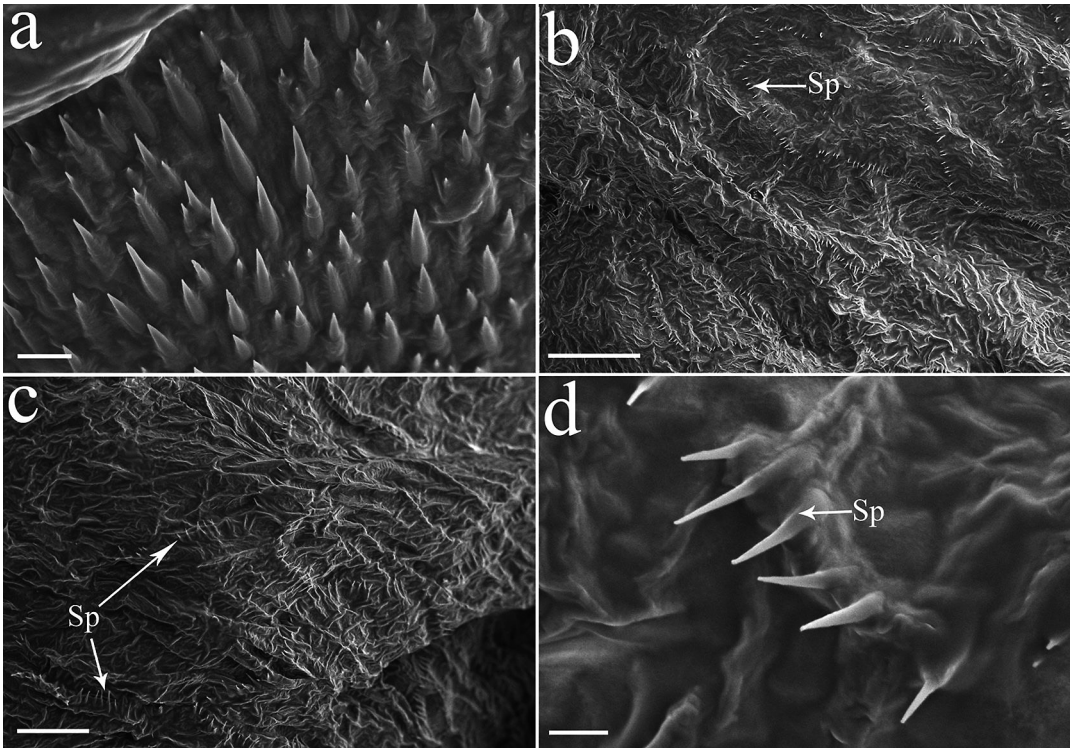

Fig. 7. SEM micrographs of two different types of spinules on cuticular intima of pylorus and pyloric valve of fifth instar larvae of *Lymantria dispar*. – a. Higher magnification of the armature of spinules in Fig. 6d. – b. Magnification of cuticular intima of anterior region of pylorus, showing abundant spinules. – c. Higher magnification of the spinules on anterior region of pylorus, showing random orientation of these spinules. – d. Higher magnification of spinules on cuticular intima of anterior region of pylorus showing almost equidistant spinules in cluster. Abbreviations: Sp, spinule. Scale bars: 4 µm in a, 40 µm in b, 20 µm in c, 2 µm in d.

other parts of the alimentary canal in terms of their musculature.

3.3. Midgut

The midgut is the largest and the longest region of the digestive tract (Fig. 1). In insects, it plays a major role in the absorption of nutrients, but also of chemical and biological insecticides (Santos *et al.* 1984, Billingsley & Lehane 1996, Barrett *et al.* 1998, Cristofoletti *et al.* 2001, Levy *et al.* 2008). The length and the diameter of the midgut may vary depending on the feeding habits of an insect species (Gillott 1995).

Unlike the foregut, the midgut of *L. dispar* larvae is an undifferentiated tube of uniform diameter throughout its entire length (Fig. 1). The midgut is covered by an internal circular and an external longitudinal layer of muscles in parallel arrays and supplied by abundant tracheae (Fig. 2d), which is similar to the condition in most other insects (Smith *et al.* 1969, Chi *et al.* 1975, Hecker 1977, Caetano 1988, Werner *et al.* 1991, Billingsley & Lehane 1996).

The epithelium is deeply transversally folded (Fig. 4a, 5a), and digested material was observed in the lumen of the midgut (Fig. 4c). Interruption of the peritrophic membrane in some regions of the midgut was observed (Fig. 5a). This may be related to the physiological renewal of the peritrophic membrane, as hypothesized by Richards and Richards (1977). The peritrophic membrane acts as a barrier to prevent food particles from coming into contact with microvilli of the midgut cells, thereby avoiding damage to the cells (Barbehenn & Kristensen 2003). A gap (ectoperitrophic space) between the epithelium and the peritrophic membrane was observed (Fig. 5a). Three types of cells distributed along the extension of the epithelium were identified under

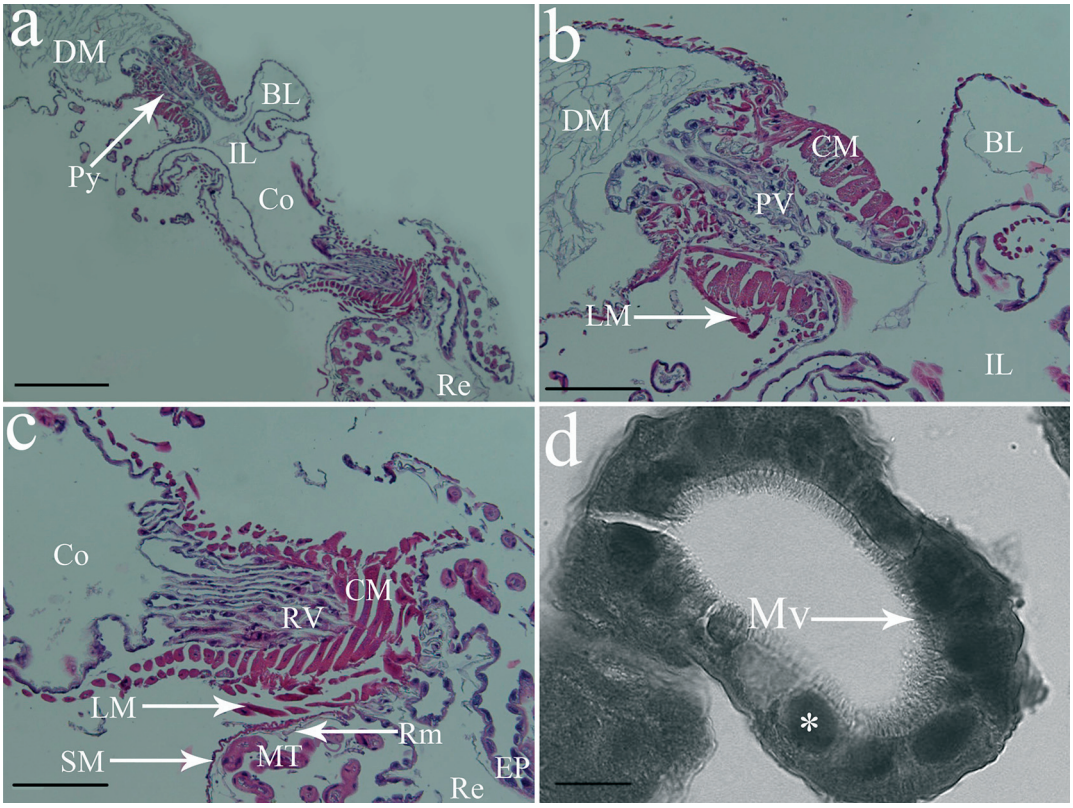

Fig. 8. Hindgut and Malpighian tubules of fifth instar larvae of *Lymantria dispar*. – a. General view of hindgut. – b. Magnification of pyloric region, showing well-developed circular muscles and pyloric valve. – c. Magnification of transition between colon and rectum, showing well-developed circular muscles and cryptonephric excretory system in detail. – d. Cross-sections of Malpighian tubules showing epithelial cells with large nuclei (white *) and microvilli in apical portion. Abbreviations: BL, bladder; CM, circular muscular layer; Co, colon; DM, digested material; EP, epithelium; IL, ileum; LM, longitudinal muscular layer; MT, Malpighian tubule; Mv, microvilli; PV, pyloric valve; Py, pylorus; Re, rectum; Rm, rectal cellular membranes; RV, rectal valve; SM, sheet muscle. Scale bars: 500 µm in a, 200 µm in b and c, 15 µm in d.

confocal microscopy: columnar, goblet, and regenerative (Fig. 4d–g). In the anterior portion of the midgut, numerous goblet cells are particularly well characterized by the presence of a chamber, limited by the plasmatic membrane projections, and with highly distinct heterochromatic basal nuclei (Fig. 4b, d–g). Similar characteristic are found in other Lepidoptera, where the goblet cells have the ability to transport potassium to maintain a high pH in the lumen, which assists the columnar cells with digestion and absorption of food (Cavalcante & Cruz-Landim 1999, Klowden 2002, Barbehenn & Kristensen 2003, De Sousa *et al.* 2009).

In the median and posterior portion of the midgut, the most frequent cells are tall columnar cells with numerous secretory granules in the apical region and characteristic brush-shaped borders (Fig. 5b, c, e, f). The regenerative cells have prominent, highly heterochromatic nuclei and indistinct cell walls and lie in groups in the base of the epithelium (Figs. 4g, 5d). According to De Sousa *et al.* (2009), the regenerative cells of insects are comparatively undifferentiated and help the midgut with ephithelium renewal. Moreover, the regenerative cells, which are usually found separated or in groups forming nests at the base of the midgut, do not only replace senescent cell, but also facilitate the gut growth during ecdysis (Cavalcante & Cruz-Landim 1999, Wanderley-Teixeira *et al.* 2006, Martins *et al.* 2006, De Sousa *et al.* 2009).

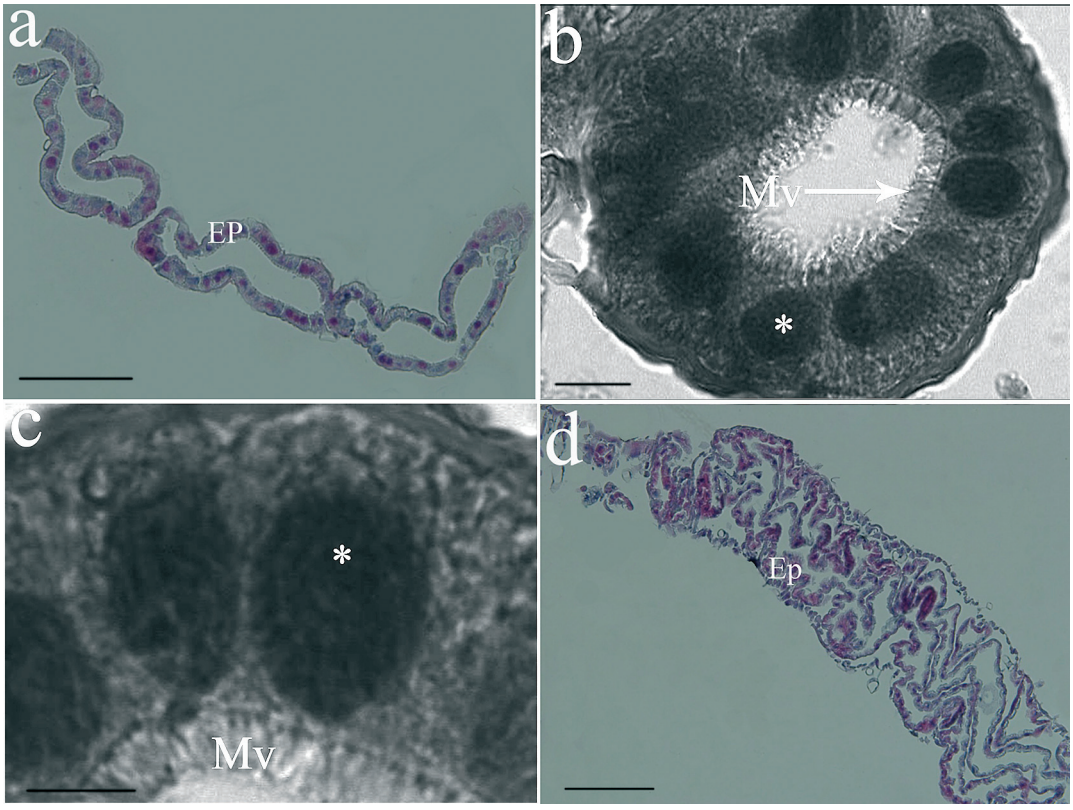

Fig. 9. Hindgut and Malpighian tubules of fifth instar larvae of *Lymantria dispar*. – a. Longitudinal section of a Malpighian tubule. – b. Cross-sections of Malpighian tubules showing epithelial cells with large nuclei (white *) and microvilli in apical portion. – c. Highly magnified cross-section of a Malpighian tubule. – d. General view of deeply longitudinally folded epithelium of hindgut. Abbreviations: EP, epithelium; Mv, microvilli. Scale bars: 100 µm in a and d, 10 µm in b, 5 µm in c.

3.4. Hindgut

The hindgut is the last and most complex region of the alimentary canal, formed sequentially from anterior to posterior by the pylorus, ileum, colon, and rectum (Figs. 1, 6a). A short posterior part following the caudal end of the midgut marks the beginning of the hindgut. It belongs to the pylorus and differs from the midgut by the absence of transverse folds, (Figs. 1, 6a, b). In this region, numerous spinules with random orientations can be seen on the cuticular intima of the pylorus (Fig. 7b, c). These spinules are almost equidistantly arranged in clusters (Fig. 7d). In the central part of the pylorus, which constitutes the pyloric valve, abundant spinules can also be observed, which are regularly arranged in large clusters different from those on the anterior region of the pylorus (Figs. 6c–d, 7a). While termites and cockroaches are known to host a rich bacterial flora attached to their hindgut spinules, and these bacteria cannot be easily washed away during specimen preparation (Bracke *et al.*, 1979), the hindgut spinules of gypsy moth look perfectly clean, which might facilitate to propel the feces.

The musculature in the pylorus consists of an inner circular and an outer longitudinal layer, and is very strongly developed in the pyloric valve (Fig. 8a, b). Here, the circular muscle layer helps the pyloric valve to close, while the prominent longitudinal muscle layer is responsible for the pyloric valve to open after food has been moved towards the ileum (Gillott 1995). There are two pyriform Malpighian bladders, which are translucent and project from the pylorus on the two lateral sides. This marks the beginning of the ileum (Figs. 1, 6a, b, 8a, b).

The ileum is a short tube, nearly as large as the

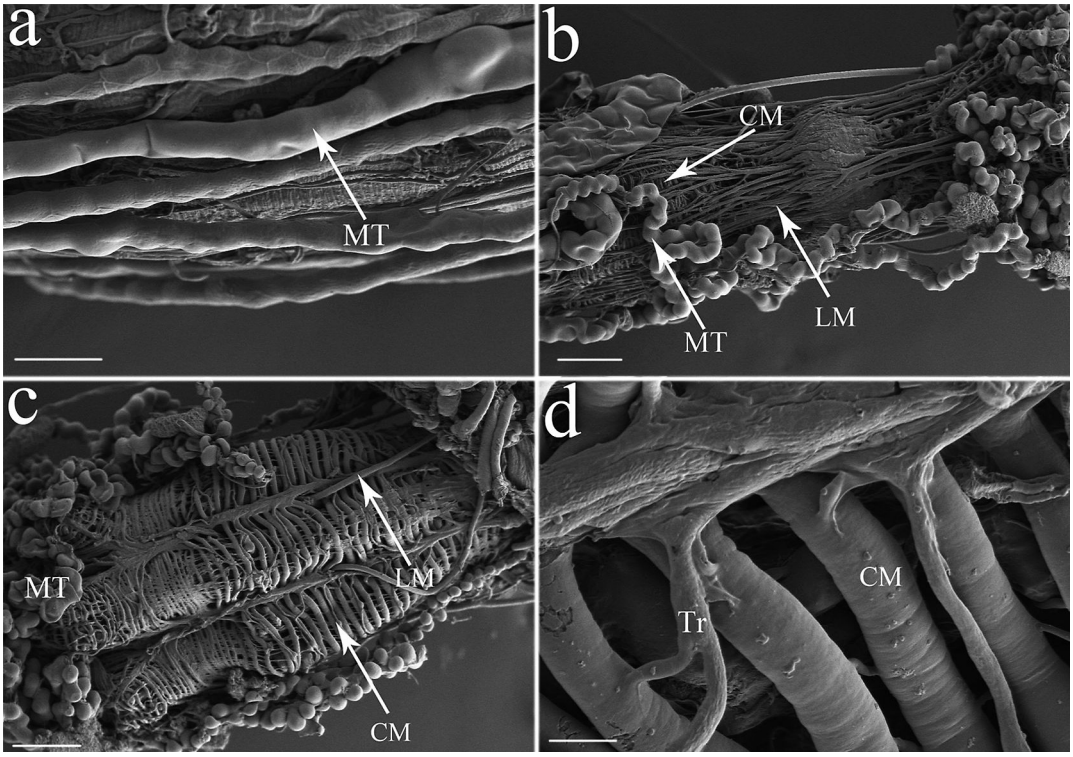

Fig. 10. SEM micrographs of hindgut and Malpighian tubules of fifth instar larvae of *Lymantria dispar*. – a, b. SEM micrographs of Malpighian tubules. – c. Ventral view of rectum. – d. Higher magnification of muscles in (c), showing tracheae connecting to muscle fibers. Abbreviations: CM, circular muscular layer; LM, longitudinal muscular layer; MT, Malpighian tubule; Tr, tracheae. Scale bars: 160 µm in a, 200 µm in b and c, 20 µm in d.

two Malpighian bladders, and more dilated than the pyloric valve. The colon is slightly wider than the ileum, but its epithelium is similar to that of the ileum (Figs. 1, 6a, 8a). Situated between the colon and rectum is the posterior sphincter region. In this region, the epithelial wall is folded longitudinally, thus forming the rectal valve, and the circular muscles of this region are as well-developed as those of the pylorus (Fig. 8a, c).

In caterpillars, the posterior sphincter is also known to bear the insertions of the dilator muscles (Judy & Gilbert 1969). These would allow contraction and dilatation of the valve, thus helping to transport feces to the rectum. In addition, the rectal valve does not allow reflux of the feces to the colon when the rectum contracts to defecate. This structure has been described for most insects, but is less developed in liquid-feeding species (Barth 1972).

The rectum appears as a distinct enlargement just posterior to the colon (Figs. 1, 6a, 8a). A thin sheet-like layer of muscles is found in the rectum wall (Fig. 8c). In general, the epithelium of the hindgut is shaped in deep longitudinal folds (Fig. 9d). The longitudinal folds are visible in a ventral view of the rectum (Fig. 10c). Feces of caterpillars are usually deposited in the form of discrete pellets, which often bear longitudinal grooves, corresponding to the longitudinal folds of the hindgut wall (Barbehenn & Kristensen 2003). The musculature of the rectum consists of an external circular and an internal longitudinal layer, with a few of the longitudinal fibers being located outside the circular layer and with minute tracheoles connected to the stretched circular fibers (Fig. 10c, d).

3.5. Silk glands

The silk glands consist of two similar and extended tubes, lying in folds in the adipose tissue

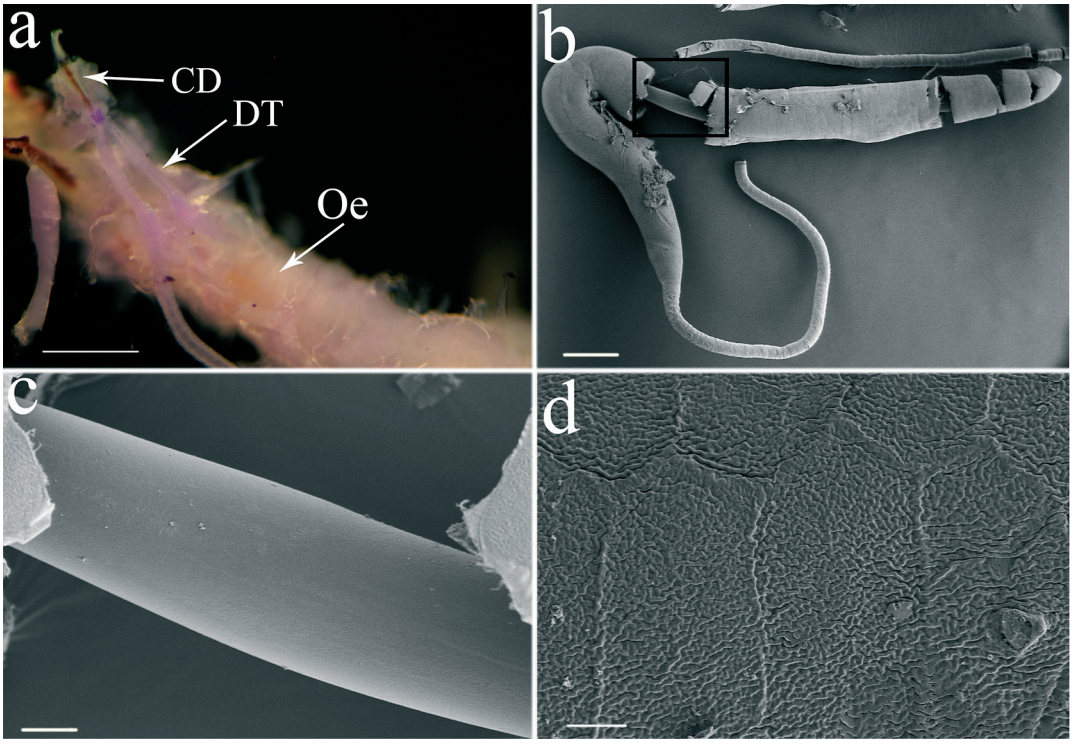

Fig. 11. Silk glands of fifth instar larvae of *Lymantria dispar*. – a. Light micrograph of anterior region of alimentary canal in ventral view, showing anterior portion of silk glands. – b. SEM micrograph of whole ruptured silk gland. – c. Higher magnification of boxed region in (b) showing cylindrical structure with smooth surface. – d. SEM micrograph of wrinkled outer surface on anterior part with larger diameter than posterior part of posterior elongated cylindrical tube. Abbreviations: CD, common duct; DT, directing tube; Oe, esophagus. Scale bars: 1 mm in a, 400 µm in b, 40 µm in c, 20 µm in d.

on either side of the digestive tract. Each gland is divided into two distinct portions, a short and narrow anterior duct – the directing tube – and the posterior elongated cylindrical tube, which varies in its diameter in anterior and posterior parts (Fig. 1). The two directing tubes unite anteriorly to form a common duct (Fig. 11a). From the ruptured part of one silk gland, a cylindrical structure with an extremely smooth external surface could be observed (Fig. 11b, c), which is presumably silk. The anterior part with larger diameter of the posterior elongated cylindrical tube has distinct wrinkled outer surface (Fig. 11d), while the surface on the posterior part with a smaller diameter is slightly wrinkled (Fig. 12a). An area of the posterior smaller part of the posterior elongated cylindrical tube, with its outer layer removed, revealed the material with rough surface (Fig. 12b). The blobs found in the glandular lumen (Fig. 12c, d) might be part of the secretory products.

Histological sections show that the glands are composed of a single layer of secretory cells around the central cavity, which is filled with silk mass (Fig. 3a, b). The basement membrane covers the gland externally (Figs. 3b, 12b, c). The intima resting on the cell walls is distinct and the cells possess prominent nuclei of varying shapes and sizes (Fig. 11b).

3.6. Malpighian tubules

Three coiled Malpighian tubules originate from each excretory chamber, here named the "bladder" (Figs. 1, 6a, b, 10a–c). Actually, as depicted by Dauberschmidt (1934), one tubule arises from each bladder, continuing in the cephalic direction, until each tubule divides after a short distance (Fig. 6b). The single pair of common ampulla-shaped Malpighian bladders inserts through the

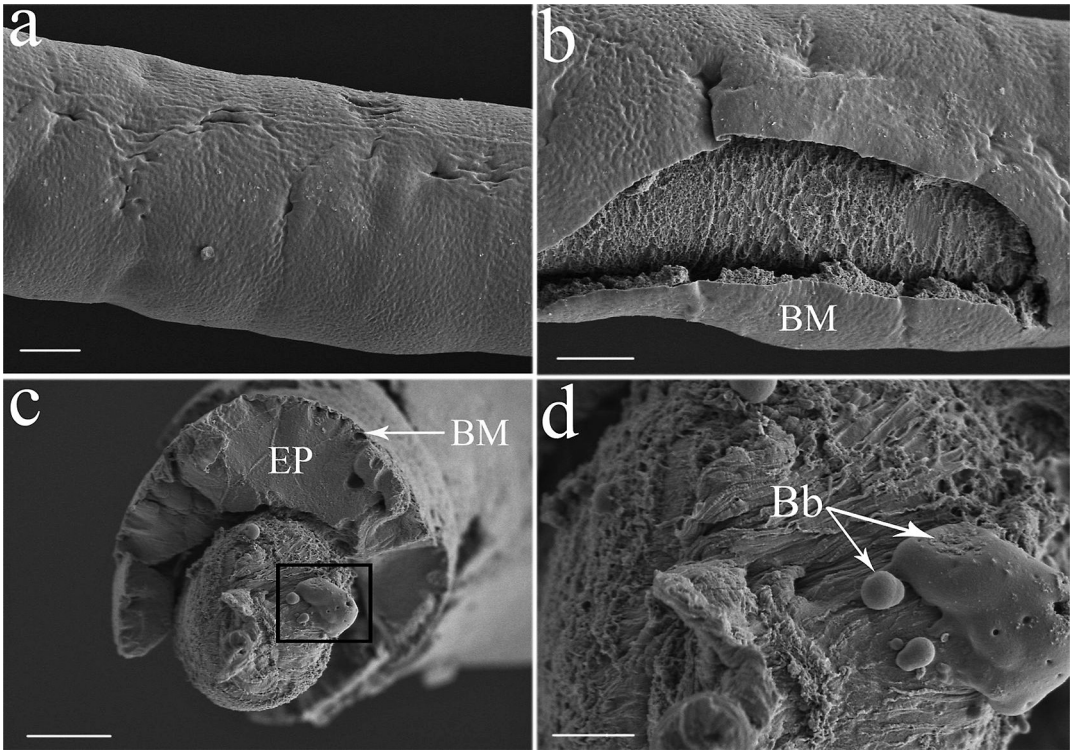

Fig. 12. SEM micrographs of silk glands of fifth instar larvae of *Lymantria dispar*. – a. Posterior part with smaller diameter than anterior part of posterior elongated cylindrical tube, showing slightly wrinkled outer surface, – b. Ruptured posterior part with smaller diameter than anterior part of the posterior elongated cylindrical tube, showing outer layer (basement membrane), – c. Cross section of posterior part with smaller diameter than anterior part of the posterior elongated cylindrical tube, showing basement membrane. – d. Higher magnification of boxed region in (c) showing fibrous material and blobs. Abbreviations: Bb, blobs; BM, basement membrane; EP, epithelium. Scale bars: 20 µm in a and b, 40 µm in c, 12 µm in d.

musculature on the ventrolateral sides of the pyloric valve (Figs. 1, 8a), marking the posterior end of the pylorus in *L. dispar*. The same condition has been described for other Lepidoptera (Standlea & Yonke 1968, Mathur 1972, Eaton 1988, Levy *et al.* 2004), Diptera (Patil & Govindan 1984), and Hymenoptera (Caetano & Overal 1984).

The two Malpighian bladders of *L. dispar* are nearly as large as the ileum (Fig. 1), thus providing a large volume to temporarily store products of excretion. White substance was observed in the bladder (Figs. 1, 6a, b), which might be uric acid and allantoic acid. This substance occupied a large area of the bladder, which might indicate a strong ability to excrete it.

The Malpighian tubules are composed of a single layer of large epithelial cells with big oval nuclei and microvilli in the apical portion, covered externally by the basement membrane (Figs. 8d, 9a–c). The epithelial cell walls of Malpighian tubules are indistinct and merged together (Fig. 9c). According to Barbehenn and Kristensen (2003), the Malpighian tubules play an important role in the detoxification of plant allelochemicals. The well-developed Malpighian tubules of *L. dispar* indicate its strong ability to detoxify these compounds, thus allowing its polyphagous diet. Barbehenn and Kristensen (2003) claimed that the Malpighian tubules excrete nitrogenous waste products, which in caterpillars are primarily in the form of uric acid, allantoic acid and/or allantoin, according to the species.

The apical portions of the Malpighian tubules are located between the epithelium of the rectum and the thin rectal cellular membranes, making up

the rectal complex that characterizes the cryptonephric excretory system (Fig. 8c). Similar characteristics have been described in other lepidopteran larvae (Drecktrah *et al.* 1966, Eaton 1988, Levy *et al.* 2008). The cryptonephric complex functions efficiently to reabsorb water from the feces, and thus is important for insects living in dry habitats to retain water (Richards & Davies 1994, Rigoni *et al.* 2004, Levy *et al.* 2008).

4. Conclusions

Our results provided a general characterization of the ultrastructure of the alimentary canal of the fifth instar larvae of *L. dispar*, for the first time combining LM, SEM and LSCM, which allow one to compare the findings with those of other insects. The ultrastructural characteristics of each region of the foregut, midgut, and hindgut were found to differ from the morphological viewpoint under the SEM and LSCM. We found a special arrangement of the intima of crop with a central ventral region recalling a ladder with numerous folds. In addition, two different types of spinules on the cuticular intima of pylorus and pyloric valve were found. These characteristics have not been found in *L. dispar* or other species earlier.

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